

**BEST AVAILABLE COPY**

# **EXHIBIT A**

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THE  
RANDOM HOUSE  
DICTIONARY  
OF THE  
ENGLISH  
LANGUAGE

Second Edition

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Unabridged

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*Dedicated to the memory of  
Jess Stein*

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r.s/uh

imp, or young scamp. 7. **out on a limb**, in a dangerous or compromising situation; vulnerable: *The company overextended itself financially and was soon out on a limb.* —**v.t.** 8. to cut the limbs from (a felled tree). [bef. 900; ME, OE *lim*; akin to ON *lim* foliage, *limb* limb, *limi* rod, *L. limus* aslant, *limen* threshold] —**limb/less**, *adj.* —**Syn.** 1. extremity. 2. See *branch*.

**limb**<sup>2</sup> (lim), *n.* 1. *Astron.* the edge of the disk of the sun, a moon, or a planet. 2. the graduated edge of a quadrant or similar instrument. 3. *Bot.* a. the upper spreading part of a gamopetalous corolla. b. the expanded portion of a petal, sepal, or leaf. [1350-1400; ME < *L. limbus*; see *LIMBUS*<sup>2</sup>, *LIMBO*<sup>1</sup>]

**lim-ba** (lim'bə), *n.* 1. an African tree, *Terminalia superba*, having yellowish-brown wood. 2. the wood of this tree. [1935-40; of uncert. orig.]

**lim-bate** (lim'bāt), *adj.* *Bot., Zool.* bordered, as a flower in which one color is surrounded by an edging of another. [1820-30; < *LL limbātus* bordered, edged. See *LIMB*<sup>2</sup>, *-ATE*<sup>1</sup>]

**limbed** (limd), *adj.* having a specified number or kind of limbs (often used in combination): a long-limbed dancer. [1275-1325; ME; see *LIMB*<sup>1</sup>, *-ED*<sup>2</sup>]

**lim-ber**<sup>1</sup> (lim'ber), *adj.* 1. characterized by ease in bending the body; supple; lithe. 2. bending readily; flexible; pliant. —**v.t.** 3. to make oneself limber (usually fol. by up): to limber up before the game. —**v.t.** 4. to make (something) limber (usually fol. by up): She tried to limber up her wits before the exam. [1555-65; perh. akin to *LIMB*<sup>2</sup>] —**lim/ber-ly**, *adv.* —**lim/ber-ness**, *n.* —**Syn.** 2. pliable. See *flexible*. —**Ant.** 1. 2. stiff. 2. rigid, unbending.

**lim-ber**<sup>2</sup> (lim'ber), *Mil.* —*n.* 1. a two-wheeled vehicle, originally pulled by four or six horses, behind which is towed a field gun or caisson. —**v.t.** 2. to attach the limber to (a gun) in preparation for moving away (sometimes fol. by up). —**v.i.** 3. to attach a limber to a gun (usually fol. by up). [1400-50; late ME *lymo(u)r* pole of a vehicle. See *LIMB*<sup>1</sup>, *-ER*<sup>1</sup>]

**lim-ber**<sup>3</sup> (lim'ber), *n.* Usually, *limbers*. *Naut.* a passage or gutter in which seepage collects to be pumped away, located on each side of a central keelson; bilge. [1620-30; perh. < *F lumière* hole, light < *LL luminaria*; see *LUMINARIA*]

**lim-ber hole**<sup>1</sup>, *Naut.* any of a series of holes pierced through a frame or floor to allow the passage of accumulated moisture. [1620-30]

**lim-ber-neck** (lim'ber nek'), *n.* *Vet. Pathol.* a fatal infection of botulism affecting birds, esp. chickens and ducks, characterized by weakness of the neck muscles and inability to eat. [1905-10; *LIMBER*<sup>1</sup> + *NECK*]

**lim/ber pine**<sup>1</sup>, a pine, *Pinus flexilis*, of western North America, having light, soft wood used locally for railroad ties, poles, fuel, etc.

**lim-bic** (lim'bik), *adj.* pertaining to or of the nature of a limb or border; marginal. [1880-85; *LIMB*(us)<sup>2</sup> + *-ic*]

**lim/bic sys-tem**, *Anat.* a ring of interconnected structures in the midline of the brain around the hypothalamus, involved with emotion and memory and with homeostatic regulatory systems. [1950-55]

**lim-bo**<sup>1</sup> (lim'bō), *n., pl. -bos*. 1. (often cap.) *Rom. Cath. Theol.* a region on the border of hell or heaven, serving as the abode after death of unbaptized infants (*lim/bo of in/fants*) and of the righteous who died before the coming of Christ (*lim/bo of the fa/thers* or *lim/bo of the pa/triarchs*). 2. a place or state of oblivion to which persons or things are regarded as being relegated when cast aside, forgotten, past, or out of date: *My youthful hopes are in the limbo of lost dreams.* 3. an intermediate, transitional, or midway state or place. 4. a place or state of imprisonment or confinement. [1300-50; ME, from ML phrase in *limbō* on hell's border (*L*: on the edge), equiv. to *in* on + *limbō*, abl. of *limbus* edge, border (*L*), place bordering on hell (*ML*); see *LIMBUS*<sup>1</sup>]

**lim-bo**<sup>2</sup> (lim'bō), *n., pl. -bos*. a dance from the West Indies, originally for men only, in which the dancer bends backward from the knees and moves with a shuffling step under a horizontal bar that is lowered after each successive pass. [1955-60; cf. Jamaican *E limba* to bend, easily bending; see *LIMBER*<sup>1</sup>]

**Lim-bourg** (*Fr.* lan bōōr'), *n.* See under *Limburg*.

**Lim-burg** (lim'būrg; *Du.* lim'bērk), *n.* a medieval duchy in W Europe; now divided into a province in the SE Netherlands (*Limburg*) and a province in NE Belgium (*Limbourg*).

**Lim-burg-er** (lim'būrg'ər), *n.* a variety of soft white cheese of strong odor and flavor. Also called *Lim/burger cheese*; *Lim/burg cheese*. [1810-20; named after *LIMBURG*; see *-ER*<sup>1</sup>]

**lim-bus**<sup>1</sup> (lim'bəs), *n., pl. -bi* (-bi). *limbo*<sup>1</sup>. [1400-50; late ME < *ML; L. LIMBUS*<sup>1</sup>]

**lim-bus**<sup>2</sup> (lim'bəs), *n., pl. -bi* (-bi) (*NL*). *Anat., Zool.* a border, edge, or limb. [1665-75; < *BL, L*]

**lime**<sup>1</sup> (lim), *n., v., limed, lim-ing.* —*n.* 1. Also called *burnt lime, calcium oxide, caustic lime, calx, quicklime*. a white or grayish-white, odorless, lumpy, very slightly water-soluble solid, CaO, that when combined with water forms calcium hydroxide (*slaked lime*), obtained from calcium carbonate, limestone, or oyster shells; used chiefly in mortars, plasters, and cements, in bleaching powder, and in the manufacture of steel, paper, glass, and various chemicals of calcium. 2. a calcium compound for improving crops grown in soils deficient in lime. 3. birdlime. —**v.t.** 4. to treat (soil) with lime or compounds of calcium. 5. to smear (twigs, branches, etc.) with birdlime. 6. to catch with or as if with birdlime. 7. to paint or cover (a surface) with a composition of lime and water; whitewash: *The government buildings were freshly limed.* [bef. 900; ME, OE *lim*; < *D. lim*, *G. Leim*, ON *lim* glue, *L. limus* slime; akin to *LOAM*] —**lime/less**, *adj.* —**lime/like**<sup>1</sup>, *adj.*

**lime**<sup>2</sup> (lim), *n.* 1. the small, greenish-yellow, acid fruit

of a citrus tree, *Citrus aurantifolia*, allied to the lemon. 2. the tree that bears this fruit. 3. greenish yellow. —**adj.** 4. of the color lime. 5. of or made with limes. [1615-25; < *Sp. lima* < *Ar. limah*, *lim* citrus fruit < *Pers. limūn* (n); cf. *LEMON*] —**lime/less**, *adj.* —**lime/like**<sup>2</sup>, *adj.*

**lime**<sup>3</sup> (lim), *n.* the European linden, *Tilia europaea*. [1615-25; unexplained var. of obs. *line*, *lind*, *ME, OE line*. See *LINDEN*]

**lime**<sup>4</sup> (lim), *n.* *Informal.* limelight. [shortened form]

**lime-ade** (lim'ad', lim'ad'), *n.* a beverage consisting of lime juice, a sweetener, and plain or carbonated water. [1890-95; *LIME*<sup>2</sup> + *-ADE*<sup>1</sup>]

**lime/ burn'er**, a person who makes lime by burning or calcining limestone, shells, etc. [1300-50; ME]

**lime/ glass**<sup>1</sup>, inexpensive glass containing a large proportion of lime, used for making cheap glasses, windowpanes, etc. [1905-10]

**Lime-house** (lim'hous'), *n.* a dock district in the East End of London, England, once notorious for its squalor; formerly a Chinese quarter.

**lime/ hy/dr-ate**. See *slaked lime*.

**lime-juic'er** (lim'jō'sər), *n.* *Slang.* 1. a British person. 2. a British sailor. [1855-60; so called because British sailors were required by law to drink lime juice to ward off scurvy]

**lime-kiln** (lim'kil', -kiln'), *n.* a kiln or furnace for making lime by calcining limestone or shells. [1250-1300; *ME limkilne*. See *LIME*<sup>2</sup>, *KILN*]

**lime-light** (lim'lit'), *n.* 1. *Theat.* a. (formerly) a lighting unit for spotlighting the front of the stage, producing illumination by means of a flame of mixed gases directed at a cylinder of lime and having a special lens for concentrating the light in a strong beam. b. the light so produced. c. *Chiefly Brit.* a lighting unit, esp. a spotlight. 2. the center of public attention, interest, observation, or notoriety: *He seems fond of the limelight.* [1820-30; *LIME*<sup>2</sup> + *LIGHT*<sup>1</sup>] —**lime/light'er**, *n.*

**lime/ lin/iment**, *Pharm.* See *carroll oil*. [1875-80]

**lim-en** (li'mən), *n., pl. li-mens, lim-i-na* (lim'ə nā). *Psychol.* threshold (def. 4). [1890-95; < *L. limen*]

**lime-qu-ot** (lim'kwot'), *n.* 1. a hybrid citrus tree produced by crossing the lime and the kumquat. 2. the tart, pale yellow fruit of this tree. [*LIME*<sup>2</sup> + (*KUM*)*QUAT*]

**lim-er-ick** (lim'ər ik), *n.* a kind of humorous verse of five lines, in which the first, second, and fifth lines rhyme with each other, and the third and fourth lines, which are shorter, form a rhymed couplet. [1895-1900; after *Limerick*; allegedly from social gatherings where the group sang "Will you come up to Limerick?" after each set of verses, extemporized in turn by the members of the party]

**Lim-er-ick** (lim'ər ik), *n.* 1. a county in N Munster, in the SW Republic of Ireland. 100,865; 1037 sq. mi. (2686 sq. km). 2. its county seat; a seaport at the head of the Shannon estuary. 60,721. 3. *Angling.* a fishhook having a sharp bend below the barb.

**lim-es** (li'mēs), *n., pl. lim-i-tes* (lim'i tēz'). 1. a boundary, esp. the fortified border or frontier of a country. 2. (cap.) See *Siegfried line*. 3. an ancient Roman frontier fortification. [1530-40; < *L. limēs*; see *LIMIT*]

**lime-stone** (lim'stōn'), *n.* a sedimentary rock consisting predominantly of calcium carbonate, varieties of which are formed from the skeletons of marine microorganisms and coral; used as a building stone and in the manufacture of lime. Cf. *marble*. [1515-25; *LIME*<sup>2</sup> + *STONE*]

**lime-stone let/tuce**, a variety of lettuce derived from Bibb lettuce.

**lime/ sul/fur**, *Chem.* a mixture of lime and sulfur that has been boiled in water; used in powdered form or in aqueous solution as an insecticide, a fungicide, and a sheep dip. Also, *lime/ sul/phur*. [1905-10]

**lime/ tree**<sup>1</sup>, a linden or basswood. [1615-25]

**lime/ twig**<sup>1</sup>, 1. a twig smeared with birdlime to catch birds. 2. a snare or trap. [1400-50; late ME]

**lime-wa-ter** (lim'wō'tər, -wō'tər), *n.* 1. an aqueous solution of slaked lime, used in medicine, antacids, and lotions, and to absorb carbon dioxide from the air. 2. water containing naturally an unusual amount of calcium carbonate or calcium sulfate. [1660-70; *LIME*<sup>2</sup> + *WATER*]

**lime-wood** (lim'wōd'), *n.* the wood of a linden. [1725-35; *LIME*<sup>2</sup> + *WOOD*<sup>1</sup>]

**lim-ey** (li'mē), *n., pl. -eys*, *adj.* *Slang* (sometimes disparaging and offensive). —*n.* 1. a British sailor. 2. a British person. 3. a British ship. —**adj.** 4. British. [1885-90; see *LIME-JUICER*, *-Y*]

**Lim/ Fjord** (lēm), a fjord in N Denmark running E from the North Sea to the Kattegat. ab. 110 mi. (175 km) long.

**lim-mic-o-line** (li mik'ə līn', -līn), *adj.* shore-inhabiting, or of pertaining to numerous birds of the families Charadriidae, comprising the plovers, and Scolopacidae, comprising the sandpipers. [1870-75; < *LL limicol(a)* mud-dweller + *-INE*; see *LIME*<sup>2</sup>, *-COLINE*]

**lim-mic-o-lous** (li mik'ə lōs), *adj.* dwelling in mud or muddy regions. [1885-90; < *LL limicol(a)* mud-dweller + *-OUS*; see *LIME*<sup>2</sup>, *-COLOUS*]

**lim-i-nal** (lim'ə nəl, li'mā-), *adj.* *Psychol.* of pertaining to, or situated at the limen. [1880-85; < *L. limin-* (s. of *limen*) threshold + *-AL*]

**lim-i-nal-i-ty** (lim'ə nəl'i tē), *n.* *Anthropol.* the transitional period or phase of a rite of passage, during which the participant lacks social status or rank, remains anonymous, shows obedience and humility, and follows prescribed forms of conduct, dress, etc. [< *L. limin-* (s. of *limen*) threshold + *-AL* + *-ITY*]

**lim-it** (lim'it), *n.* 1. the final, utmost, or furthest boundary or point as to extent, amount, continuance

procedure, etc.: *the limit of his experience; the limit of vision.* 2. a boundary or bound, as of a country, area, or district. 3. *Math.* a. a number such that the value of a given function remains arbitrarily close to this number when the independent variable is sufficiently close to a specified point or is sufficiently large. The limit of  $1/x$  is zero as  $x$  approaches infinity; the limit of  $(x - 1)^2$  is zero as  $x$  approaches 1. b. a number such that the absolute value of the difference between terms of a given sequence and the number approaches zero as the index of the terms increases to infinity. c. one of two numbers affixed to the integration symbol for a definite integral, indicating the interval or region over which the integration is taking place and substituted in a primitive, if one exists, to evaluate the integral. 4. *limits*, the premises or region enclosed within boundaries: *We found them on school limits after hours.* 5. *Games.* the maximum sum by which a bet may be raised at any one time. 6. *the limit*, *Informal.* something or someone that exasperates, delights, etc., to an extreme degree: *You have made errors before, but this is the limit.* —**v.t.** 7. to restrict by or as if by establishing limits (usually fol. by to): *Please limit answers to 25 words.* 8. to confine or keep within limits: *to limit expenditures.* 9. *Law.* to fix or assign definitely or specifically. [1325-75; ME *lymit* < *L. limit-* (s. of *limes*) boundary, path between fields] —**lim/it-a-ble**, *adj.* —**lim/it-a-ble-ness**, *n.* —**lim/it-er**, *n.* —**Syn.** 2. confine, frontier, border. 8. restrain, bound.

**lim-i-tar-y** (lim'i ter'ē), *adj.* 1. of, pertaining to, or serving as a limit. 2. *Archaic.* subject to limits; limited. [1610-20; *LIMIT* + *-ARY*]

**lim-i-ta-tion** (lim'i tā'shən), *n.* 1. a limiting condition; restrictive weakness; lack of capacity; inability or handicap: *He knows his limitations as a writer.* 2. something that limits; a limit or bound; restriction: *an arms limitation; a limitation on imports.* 3. the act of limiting. 4. the state of being limited. 5. *Law.* the assignment, as by statute, of a period of time within which an action must be brought, or the period of time assigned: *a statute of limitations.* [1350-1400; ME *lymitacion* < *L. limitatiōn-* (s. of *limitatiō*) a bounding, equiv. to *limitatiō* (p. of *limitare* to enclose within boundaries; see *LIMIT*, *-ATE*<sup>1</sup>) + *-iōn* (-iōn)]

**lim-i-ta-tive** (lim'i tā'tiv), *adj.* limiting; restrictive. [1520-30; < *ML. limitatiuus*. See *LIMITATION*, *-IVE*]

**lim-it-ed** (lim'it id), *adj.* 1. confined within limits; restricted or circumscribed: *limited space; limited resources.* 2. restricted with reference to governing powers by limitations prescribed in laws and in a constitution: *a limited monarch.* 3. characterized by an inability to think imaginatively or independently; lacking originality or scope; narrow: *a rather limited intelligence.* 4. *Chiefly Brit.* a. responsible for the debts of a company only to a specified amount proportionate to the percentage of stock held. b. (of a business firm) owned by stockholders, each having a restricted liability for the company's debts. c. (usually cap.) incorporated; Inc. *Abbr.: Ltd.* 5. (of railroad trains, buses, etc.) making only a limited number of stops en route. —**n.** 6. a limited train, bus, etc. [1545-55; *LIMIT* + *-ED*<sup>2</sup>] —**lim/it-ed-ly**, *adv.* —**lim/it-ed-ness**, *n.*

**lim/it-ed ac/cess high-way**, expressway. [1940-45]

**lim/it-ed com/pany**, *Brit.* a company in which the shareholders cannot be assessed for debts of the company beyond the sum they still have invested in the company. Also called *lim/it-ed-li-a-bil-i-ty com/pany* (lim'i tid li'ə bil'i tē). [1850-55]

**lim/it-ed di-vorce**<sup>1</sup>, *Law.* See *Judicial separation*.

**lim/it-ed edi-tion**, an edition, as of a book or lithograph, limited to a specified small number of copies. [1900-05] —**lim/it-ed-e-di-tion**, *adj.*

**lim/it-ed lib/er-ty**, a liability restricted by law or contract, as the liability of owners of shares in a corporation or limited company, or that of a special partner. [1850-55]

**lim/it-ed mon/archy**, a monarchy that is limited by laws and a constitution. [1825-35]

**lim/it-ed or/der**. See *limit order*.

**lim/it-ed part/ner**. See *special partner*. [1905-10]

**lim/it-ed part/nership**, a partnership formed by at least one general partner and at least one special partner. Also called *special partnership*. Cf. *general partnership*. [1905-10]

**lim/it-ed-pay/ment life/ insur/ance** (lim'i tid-pā'mənt), a form of life insurance for which premiums are paid for a designated number of years.

**lim/it-ed pol/icy**, *Insurance.* a policy that covers only certain types of losses within an area of risks.

**lim/it-ed-slip/ differ-en-tial** (lim'i tid slip'), an automotive differential that can transfer power from a wheel that has lost traction to one that has not.

**lim/it-ed war**<sup>1</sup>, 1. a war conducted with less than a nation's total resources and restricted in aim to less than total defeat of the enemy. 2. a war restricted to a relatively small area of the world and involving few warring nations. [1935-40]

**lim-it-er** (lim'i tər), *n.* 1. a person or thing that limits. 2. *Electronics.* a device or circuit for limiting the amplitude of a radio, telephone, or recording signal to some predetermined level. [1350-1400; *LIMIT* + *-ER*<sup>2</sup>; r. ME *limitour* (see *-OR*<sup>2</sup>)]

**lim-i-tes** (lim'i tēz'), *n.* pl. of *limes*.

**lim-it-ing** (lim'i ting), *adj.* 1. serving to restrict or restrain; restrictive; confining. 2. *Gram.* of the nature of a limiting adjective or a restrictive clause. [1570-80; *LIMIT* + *-ING*<sup>2</sup>]

CONCISE PRONUNCIATION KEY: act, cāpe, dāre, pārt; set, equal; if, ice; ox, ūer; order, oil, bōat, bōat; out, up, ūrge; child; sing; shoe; thin; that; zh as in treasure. a = a as in alone, e as in system, i as in easily, o as in gallop, u as in circus; \* as in fire (fīr), hour (aūr), and n can serve as syllabic consonants, as in cradle (krād'l), and button (but'n). See the full key inside the front cover.

## **EXHIBIT B**

# PROTEIN PURIFICATION

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## Principles, High-Resolution Methods, and Applications

SECOND EDITION

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Bio-Sil SEC	5	5, 10	20–1,000						
Silica	5	5, 10	20–1,000						
DuPont GF-250 <sup>a</sup>	6	4	10–250	14–100	0.52	0.78	0.40	3–8.5	
DuPont GF-450	6		25–900					3–8.5	
PL-GFC 300 Å	7	8, 10	1–700						
Polymer									
Bulk media									
Agarose/ Superdex 30	1	34	<10			1.8		1–14	
Superdex 75	1	34	3–70			2.1		1–14	
Agarose/ Superdex 200 prep grade	1	34	100–600			2.2		1–14	
Fractogel TSK HW-55S <sup>c</sup>	2	25–40	1–1,000	0.2–100	0.96	2.17	0.31	1–14	25 mM Tris-HCl + 0.3 M NaCl, pH 7.5 <sup>27</sup>
Vinyl polymer									0.05 M phosphate + 0.15 M NaCl, pH 7.0 <sup>28</sup>
Supersore 12 prep grade	1	20–40	1–2,000	1–600	0.52	1.86	0.33	1–14	
Supersore 6 prep grade	1	20–40	5–40,000	5–10,000	0.97	2.23	0.30	1–14	

\* Data as stated by the supplier: 1, Pharmacia, Biotech (Uppsala, Sweden); 2, Toyo Soda (Tokyo, Japan); 3, SynChrom (Linden, IN); 4, Waters (Milford, MA); 5, Bio-Rad (Hercules, CA); 6, DuPont (Wilmington, DE); 7, Polymer Laboratories (Shropshire, UK).

<sup>b</sup> For globular proteins, calculated from  $0.1 \leq K_D \leq 0.9$ .

<sup>c</sup>Data from Ref. 18.

<sup>d</sup> Data calculated from Ref. 22.

• Toyoparl HW type.

TABLE 3-2. Characteristics of Some Media for High-resolution Gel Filtration of Proteins and Peptides<sup>a</sup>

TABLE 3-2. Characteristics of Some Media for High-Resolution Gel Permeation Chromatography

Media	Type	Supplier	Particle Size ( $\mu\text{m}$ )	Fractionation		Operation Range <sup>b</sup> ( $M_r \times 10^{-3}$ )	Molecular Mass Selectivity ( $-\Delta K_D/\Delta \log M_r$ )	Particle Porosity [ $V_p/(V_i + V_p)$ ]	Permeability ( $V_p/V_o$ )	Void Fraction ( $V_o/V_o$ )	pH Stability	Buffer Composition
				Range of Globular Solutes ( $M_r \times 10^{-3}$ )								
Prepacked columns												
Superdex Peptide	Agarose/dextran	1	13	0.1-7					1.2		1-14	
Superdex 75	Agarose/dextran	1	13	3-70					1.6		1-14	
Superdex 200	Agarose/dextran	1	13	10-600					1.7		1-14	
Superose 12	Agarose	1	10	1-2,000		0.5-600	0.28	0.84	1.84	0.30	1-14	0.05 M phosphate +
Superose 6	Agarose	1	13	5-40,000		5-10,000	0.23	0.93	1.87	0.33	1-14	0.15 M NaCl, pH 7.0 <sup>a</sup>
TSK SW 2000	Silica	2	10	0.5-60		0.7-100	0.52	0.66	0.95	0.39	2.5-7.5	0.05 M Tris-HCl +
TSK SW 3000	Silica	2	10	1-300		2-400	0.43	0.81	1.33	0.38	2.5-7.5	0.5 M Na <sub>2</sub> CO <sub>3</sub> , pH 7.5 <sup>a</sup>
TSK SW 4000	Silica	2	13	5-1,000		6-10,000	0.28	0.89	1.40	0.39	2.5-7.5	
Synchropak <sup>c</sup> GPC 100	Silica	3	10	3-630			0.44	0.79	1.23	0.39		0.1-0.6 M phosphate, pH 7 <sup>a</sup>
Waters I-125 <sup>c</sup>	Silica	4	10	0.8-450			0.36	0.55	0.92	0.38		0.08 M phosphate + 0.32 M NaCl in 20% ethanol <sup>a</sup>



gen bonding, and the nature of the buffer ions can also influence the separation. Similarly, separation in chromatofocusing reflects not only the pI of a protein, but also the shape of the titration curve in the vicinity of the pI. Chromatofocusing, which in fact is a special form of IEC, is treated in Chapter 5.

Even though IEC is an established technique in most biochemical laboratories, little is known about the fundamental mechanism behind protein binding to charged surfaces. Discussions regarding different adsorption models can be found in Refs. 2, 3, and 4.

The first use of an ion exchanger in protein chemistry may have been for the removal of pectinmethylesterase from a preparation of pectinpolygalacturonase. The contaminant was adsorbed to a polystyrene cation exchanger (Amberlite IR-100, sulfonic acid type).<sup>5</sup> Some years later the styrenemethacrylic acid resin Amberlite IRC-50 was used for successful chromatographies of a number of basic proteins,<sup>6</sup> such as cytochrome c,<sup>7</sup> ribonuclease,<sup>8,9</sup> and lysozyme.<sup>10,11</sup> Methacrylic acid resins have a high  $pK_a$  of 6.5 (at ionic strength 0.1 M) and are therefore suited for basic proteins. Because of their very hydrophobic matrices and low capacity, polystyrene resins are not suited for protein chromatography (see Section 4.4).

The introduction of ion exchangers with hydrophilic and macroporous matrices in the middle of the mid-1950s by Sober and Peterson<sup>12-14</sup> extended ion-exchange chromatography to most proteins. They synthesized carboxymethyl (CM) and diethylaminoethyl (DEAE) derivatives of cellulose. Adsorption of proteins by forces other than electrostatic is low and they have a high capacity, as the macroporous structure renders ion-exchange groups in the interior of the particles accessible to proteins. Since then, a number of chromatographic media, in particular beaded ones, have been synthesized. These include gels based on cross-linked dextrans, cross-linked agarose, synthetic hydrophilic polymers, and small, rigid beads used in HPLC.

This chapter is devoted mainly to practical aspects of ion-exchange chromatography. Hydroxyapatite chromatography will also be briefly treated, as it may be regarded as a variant of ion-exchange chromatography. The chapter concludes with some examples of practical applications.

## 4.2 THE ION-EXCHANGE PROCESS

### 4.2.1 Fundamental Concepts

The basis for IEC is the competition between ions of interest and other ions for oppositely charged groups on an ion exchanger. The interaction between small molecules and an ion exchanger depends on the net charge, as illustrated in Fig. 4-1, and the ionic strength of the medium. When the concentration of competing ions is low, the ions of interest bind to the ion exchanger, whereas when it is high, they are desorbed. The interaction between a protein and an ion exchanger depends not only on the net charge and the ionic strength, but

$A^- A^+$



**FIGURE 4-1.** Principle charges ( $A^{3+}$ ) are adsorbed; those with no net charge pass through the column and the separation occurs. With protein molecules, the separation (see text)

also on the surface particular ions in the of the ion exchanger. The interaction  $F$  is given by Coulom

where  $D$  is the dielectric constant, about 80, and static attraction between phobic environment. thick layer of solvent a direct interaction, inhibitor or substrate

influence the separation, only the pI of a protein, of the pI. Chromatofocusing in Chapter 5.

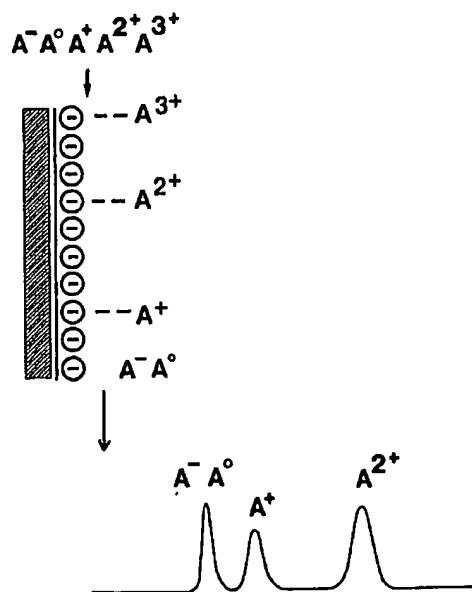
most biochemical laboratory behind protein binding adsorption models

istry may have been for on of pectinpolygalactosyl cation exchanger later the styrenemethacrylate chromatographies of c,<sup>7</sup> ribonuclease,<sup>8,9</sup> and of 6.5 (at ionic strength 0.1). Because of their very low ionic strength, these resins are not suited for

philic and macroporous ion exchange resins. Peterson<sup>12-14</sup> extended the use of synthesized carboxylic acids of cellulose. Adsorption of proteins to these resins is high and they have a high capacity for ion-exchange groups in the resin. Then, a number of chromatographic resins have been synthesized. These include agarose, synthetic ion exchange HPLC.

of ion-exchange chromatography be briefly treated, as it is a chromatography. The chapter on ion-exchange chromatography.

of interest and other ions. The interaction between a protein and an ion exchanger, as illustrated in Figure 4-1, depends on the net charge of the protein, the concentration of the ion exchanger, whereas the interaction between a protein and an ion exchanger depends on the ionic strength, but



**FIGURE 4-1.** Principle of ion-exchange chromatography. Species with several positive charges ( $A^{3+}$ ) are adsorbed to the column; those with few charges move slowly, whereas those with no net charge or a net charge of the same sign as the adsorbent pass through the column unretained. The resulting chromatogram is shown. In this case, the separation occurred according to differences in net charge. This is valid for small molecules. With proteins, many other factors besides the net charge can contribute to the separation (see text).

also on the surface charge distribution of the protein, pH, the nature of particular ions in the solvent, additives such as organic solvents, and properties of the ion exchanger.

The interaction  $F$  between two charges  $Z_a$  and  $Z_b$  separated by a distance  $r$  is given by Coulomb's law

$$F = \frac{Z_a Z_b}{D r^2} \quad (4-1)$$

where  $D$  is the dielectric constant of the medium. Water has a high dielectric constant, about 80, and most organic solvents have about 20. Thus, the electrostatic attraction between two oppositely charged groups is higher in a hydrophobic environment. For the value of  $D$  to be valid, there must be a sufficiently thick layer of solvent molecules between the two charged groups. If there is a direct interaction, as between say an active site residue of a protein and its inhibitor or substrate, the bond is in general stronger than would be predicted

# **EXHIBIT C**

DL

CAMY EW

# Structure and Assembly of Adenoviruses

LENNART PHILIPSON

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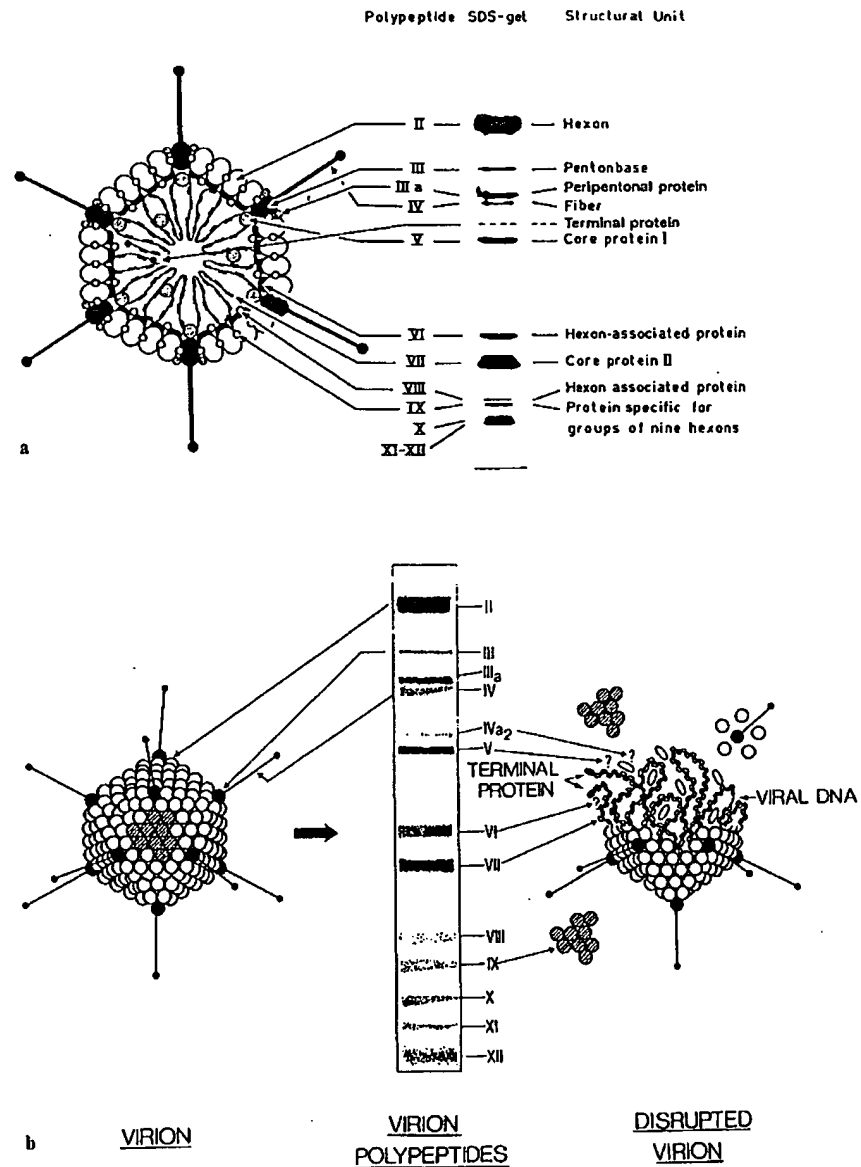


Fig. 4a, b. Two tentative models for the location of the proteins in the Ad2 virion. The polypeptide composition of the virion proteins are shown in a stained exponential 10–16% SDS–polyacrylamide gel, and the evidence for the location of the different protein moieties is summarized in the text. A covalently linked terminal protein is also indicated in the figure, but cannot be seen in a protein gel, and the corresponding polypeptide band is therefore indicated with a broken line. a A model based on protein-protein cross-linkage studies. [Modified from EVERITT et al. (1975)]. b A schematic model. [RUSSELL and PRECIOUS (1982)]

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